"BIO-CONTROL POTENTIAL OF *PSEUDOMONAS FLUORESCENS* ON PEA (*PISUM SATIVUM* L.) IN MID HILL CONDITIONS OF HIMACHAL PRADESH."

SYNOPSIS OF THESIS

Submitted By

Aishwarya

Registration No : AU21AGPhD0076

Under the Supervision of

Dr. Ajay Kumar Gautam

In Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

SCHOOL OF AGRICULTURE

FACULTY OF AGRICULTURE

ABHILASHI UNIVERSITY

CHAIL CHOWK, CHACHYOT MANDI (H.P.)-175028

SEPTEMBER 2023

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Title approved 30 Sept 2023

Submitted to the

School of Agriculture
Abhilashi University Chail Chowk,
Mandi H.P.-175028, India

DECLARATION

I hearby that the dissertation synopsis entitled "**Bio-control potential of *Pseudomonas fluorescens* on Pea (*Pisum sativum* L.) in mid hill conditions of Himachal Pradesh.**" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship or fellowship of similar other titles, it has not been submitted to any other University or institution for the award of any degree or diploma.

Place: Chailchowk, Mandi (H.P)

Date:

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Recommended & Forwarded to
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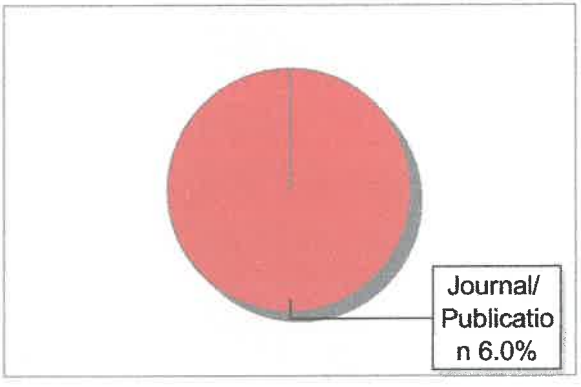

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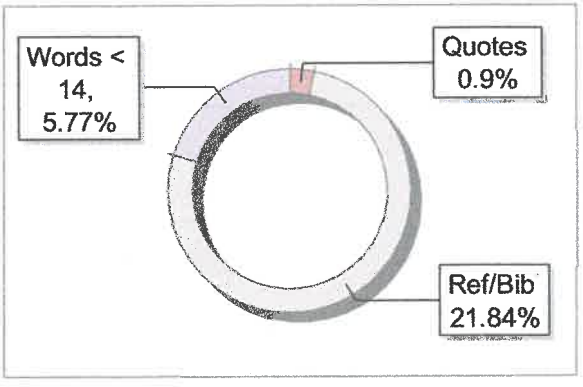

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ABHILASHI UNIVERSITY

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SCHOOL OF AGRICULTURE

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Synopsis of Thesis of Post Graduate Student

Name of the student : Aishwarya

Registration No. : AU21AGPhD0076

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2. Soil Science

Title of the research problem : "Bio-control potential of *Pseudomonas fluorescens* on Pea (*Pisum sativum* L.) in mid hill conditions of Himachal Pradesh."

1. Introduction

The ubiquitous leguminous crop known as the pea (*Pisum sativum* L.) is a member of the Fabaceae family. The name "faba" is derived from the Latin word "fabus," which simply means "beans". Germany, Italy, China, Canada, followed by India, Australia, and the United States are the major pea producing countries of the world. In addition to production, France, Canada, and Australia are major exporters of pea due to their use of over two million hectares of land for pea cultivation. The majority of India's peas are grown in Uttar Pradesh, which accounts for 49% of all pea production in the nation. The primary pea-growing states in India are Madhya Pradesh, Punjab, and Jharkhand in addition to Uttar Pradesh. Himachal Pradesh, a mountainous state in India, ranks fifth in terms of annual pea output with a total of 294.96 thousand metric tons. The crop produced in the state is thought to be very nutritive, as it contains a high proportion of

digestible proteins, carbohydrates, and fats, as well as minerals (Ca, P, and Mg) and vitamins A, B, and C. Peas are used as food either alone or combined with other vegetables (FAO 2021).

Using nitrogen fixation by *Rhizobium leguminosarum*, the legume crop pea increases soil fertility without the additional cost of additional fertilizer. Small-scale terraced fields and dispersed, fragmented landholdings characterize the agricultural landscape in hilly areas. Himachal Pradesh is renowned for its diverse climatic conditions, soil types, and agro-ecologies, as well as for having a wide range of plant health. Since it is a crop, peas are susceptible to numerous illnesses on a global scale. These illnesses may be brought on by a variety of foliar and soil-borne pathogens. According to reports, all types of diseases can impair the general growth and development of an affected crop, including peas. Some significant field pea diseases produced by several phytopathogens include soil-borne diseases including seed rot, root rot, seedling blights, Rhizoctonia root rot, Fusarium root rot, and Aphanomyces root rot, as well as foliar diseases such powdery mildew, rust, blights, and leaf spots. Diseases brought on by soil-borne pathogens result in poor crop quality and yield. The most significant soil-borne pathogens that harmed several important crops were fungi.

The principal pathogenic fungi identified to be related with pea are determined to belong to the fungal genera Phytopthora, Erysiphe, Rhizoctonia, and Fusarium (Djonovic *et al*. 2007). A wide variety of microorganisms that either promote or restrict growth are associated with the rhizosphere. Different plant growth-promoting microorganisms have been shown to reduce soil-borne diseases, enhance plant growth regulators, promote root exudation, and increase the availability of plant nutrients when they colonize the roots or are present in the rhizosphere of plants (Ahemad and Kibret, 2014).

According to reports, the PGPR includes Pseudomonas, Azospirillum, Azotobacter and Trichoderma. Through acidification, chelation, and iron-exchange processes, phosphate-solubilizing microorganisms delivered insoluble phosphate to plants that had been supplied to or already existed in the soil (Coutinho *et al*. 2012). Plant Growth Promoting Rhizobacteria (PGPR), a type of free-living soil bacterium, has been recommended to boost plant growth regulators as well as to promote root exudation, and increase the availability of plant nutrients to the plant roots (Akhtar *et al*. 2014). *Pseudomonas fluorescens* also helps in controlling and minimizing the soil-borne diseases. Similar to this, *Pseudomonas fluorescens* develops amylase to break down the polymeric compounds (Molobela *et al*. 2010).

According to studies conducted under a variety of environmental conditions, *Trichoderma spp.*, *Pseudomonas fluorescens*, and *Bacillus subtilis* are the most effective biocontrol agents against a number of plant infections (Chet 1987). According to recent reports, the combination of biocontrol agents has reduced the prevalence of disease in several plants (Latha *et al*. 2011, 2011). Numerous seed-borne fungus, including *Fusarium moniliforme*, *Fusarium oxyysporum, Rhizoctonia solani*, *Fusarium solani*, *Botryodiplodia theobromae*, and *Alternaria alternata,* are controlled by *Trichoderma spp.* in a dual culture assay (Saharan and Nehra 2011). *Trichoderma spp.* strains and antagonistic bacteria like *Pseudomonas spp*. have been shown to combine synergistically to combat plant diseases (Whipps 1997). N_2 fixation, the

solubilization of mineral phosphates and other nutrients, the capacity to create phyto hormones, vitamins, enzymes, and siderophores, as well as defense against plant diseases, are some of the ways through which PGPR promotes plant growth. In order to assess the biological control capacity of *Pseudomonas fluorescens* on pea (*Pisum sativum* L.) illnesses in mid-hill settings of Himachal Pradesh, the present study will be conducted with following objectives:

Objectives:

a) To study the effects of *Pseudomonas flourescens* on major diseases of Pea (*Pisum sativum* L.) in mid hill conditions of Himachal Pradesh,

b) To evaluate the effects of *Pseudomonas flourescens* on growth parameters of Pea (*Pisum sativum* L.) in mid hill conditions of Himachal Pradesh.

3. A Brief Resume of Work Done in Abroad, India and Himachal Pradesh

a) Work done in abroad

In an experiment on the sustainable use of natural resources, Gloria *et al*. (2006) found that conventional agriculture had a significant negative impact on soils and waters. It's critical to switch to more environmentally friendly agricultural management practices. They proposed important facets of this broad subject, such as (i) the rhizosphere as a microhabitat; (ii) the taxonomic, genetic, and ecological aspects of fluorescent Pseudomonads in the rhizosphere; (iii) mechanisms of Plant Growth Promoting and Biological Control; and (iv)the commercial use of PGPR in agriculture.

According to Sara and Diego *et al*. (2012) research on pea powdery mildew, it is an airborne illness with a global reach. The disease can reduce total biomass yield, the number of pods per plant, the number of seeds per pod, plant height, and the number of nodes, all of which can result in yield losses of 25–50%. The spread of new pathogen races that would compromise the resistance could be encouraged by expanding the cultivation areas of pea varieties carrying the same resistance gene. The use of polygenic resistance or the fusion of several important genes may increase the resistance's resilience.

Guanpeng *et al*. (2012) conducted research on the importance of fungi and the fungi community in the soil ecosystem as well as the potential for the diversity of the fungi community to serve as natural antagonists of various plant pathogens. Pseudomonas fluorescens 2P24 was isolated from the wheat rhizosphere take-all decline soils in Shandong province, China. It had strong inhibitory effects on *Rastonia solanacearum*, *Fusarium oxysporum*, and *Rhizoctonia solani*, among other pathogens. Although different enzymes were used for digestion, there was little difference. The decline of *P. fluorescens* 2P24 caused the soil fungal community to slowly recover after initially being severely shocked, according to DGGE results. There was not much

of a difference between the treatment and control four weeks later. Generally speaking, *P. fluorescens* 2P24's impact on the microbial community in the cucumber rhizosphere

Naglaa *et al*. (2018) conducted research on the root rot pathogens involved in isolation, identification, and pathogenicity testing, including *Fusarium oxysporum, Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Thielaviopsis basicola*. The results revealed that treated root pea plants had lower PG and Cx enzyme activities than untreated ones; these enzymes are thought to play a role in pathogenesis. In order to achieve the most effective disease control, additional research is required to determine the best application techniques.

Abdullah (2020) carried out an experiment to determine whether microbe fitness and plant colonization effectiveness are important for promoting plant growth, but the mechanism between plants and bacteria in the rhizosphere is not fully understood. The goal of this study was to identify and characterize a few bio-control bacteria that promote plant growth. 94 different bacteria strains were found and they were examined for their ability to promote various aspects of plant growth as well as their antagonistic traits toward various pathogenic fungi. Therefore, using biocontrol agents on alfalfa plants can effectively control the soil-born fungal pathogen.

b) Work done in India

In the Kumaon Hills of the Himalayas, Hooda *et al*. (2006) conducted a field experiment to investigate the effects of bio-control agents on the health of garden pea (*Pisum sativum*). Results regarding the efficiency of biological agents in preventing disease support the findings are the findings concur with those of Muralidharan *et al*. (2004). Verma and Dohroo (2002) and Kumar (2001) findings regarding the efficiency of bio central agents in the prevention of disease are supported by the results of this study.

Girija and Kumar (2005) conducted experiments on fluorescent Pseudomonas, a member of the plant Growth Promoting Rhizobacteria (PGPR), and reported that this is a significant group of bacteria that play a significant role in the promotion of plant growth. Many strains of *Pseudomonas fluorescens* are known to improve the promotion of plant growth and lessen the severity of various diseases. In order to promote *P. fluorescens* as a potential bio-pesticide for the supplemental biological control of numerous diseases of importance to agriculture and horticulture, future research programs will benefit from the literature reviewed in this review.

Pseudomonas fluorescens (Pf) strains have shown inconsistent performance in the past when tested under controlled and field conditions for plant growth promotion and bio-control activities, according to Yogesh *et al*. (2008) reports. The improved plant growth indicators in pea suggested that the isolates were also effective PGPRs. The fact that these isolates consistently performed under field circumstances may be related to the fact that the environments in which they were used were native to the isolates.

According to Aflaq *et al*. (2012), pea root rot is becoming a serious disease in Kashmir. *Trichoderma harzianum* showed the highest inhibition percentage of 78.60% in the dual culture

studies of the four bio-control agents, followed by *Trichoderma viride* (75.72%), *Gliocladium virens* (69.52%), and *Pseudomonas fluorescence* (68.37%). The only bio-control agent that displayed a zone of inhibition in dual culture was *P. fluorescence*. The bio-control agents used to observe the seed treatments revealed that *T. viride* (86%), *T. harzianum* (90% each), *P. fluorescence* (83%) and *G. virens* (82%) caused the highest germination percentages, while control pots only saw a germination rate of 61%.

According to Sharma *et al*. (2017) analysis, growers were able to maintain increased crop productivity by using chemical fertilizers and fungicides in conjunction with good agronomic practices. They noted that the extensive use of chemicals in the last few decades for disease and pest control had adverse effects on the environment, produced products of lower quality, and harmed consumer health. They examined whether various bio-control agents, such as fungi and bacteria, have been identified but need to be effectively adopted and developed. A new era in plant disease management has begun with the analysis of microbial communities using culture-independent molecular techniques, such as sequencing technologies and genomics data.

To identify morphological and biochemical traits under a compound microscope in *Trichoderma viride* (MH333256) and *Pseudomonas fluorescens* (Ps01), Ramasamy and Sundaram (2020) performed an experiment. Genetic DNA isolation and PCR analysis were used to confirm the phylogenetic relationships of each isolate. They noticed that when compared to the control (9 g) and Fu04 (6 g), the bio inoculants (Tr02 and Ps01) increased the plant's fresh weight (13.4 g) and inhibited the plant pathogen (Fu04).

c) Work done in Himachal Pradesh

Verma *et al.* (2018) studied a total of 574 endorhizobacterial isolates and 859 rhizobacterial isolates that were collected from various tomato-growing locations in Himachal Pradesh, India. Only 17 isolates were found to be effective phosphate solubilizers with 15 mm or more halo zone formation on PVK medium after being screened for their plant growth promoting attributes. Rhizobacteria and endorhizobacteria predominate populations varied according to sites. In the study, it was found that 52.94% of the bacteria showed inhibition against specific soil-borne pathogens, while 58.82% of the bacteria were nitrogen fixers. *Bacillus sp*., *Pseudomonas sp., Enterobacter sp., Acinetobacter sp*., and some unidentified P-solubilizing bacteria were all colonized in the tomato rhizosphere, according to morphological and biochemical characterization.

The use of plant growth promoting rhizobacteria (PGPR) in agriculture is gaining prominence globally and is viewed as a novel and potential tool for providing significant benefits, according to Singh *et al*. (2019) study on apple replant disease, which has negatively impacted apple orchards for many years and is now present in most fruit growing regions worldwide. As a result, they discovered that a few Pseudomonas sp. strains that had been

phenotypically characterized had been found to be potent and may be useful in the fields of agriculture and horticulture, particularly in the treatment of apple plants in replant problem areas.

Twenty native fluorescent pseudomonads were isolated from apple replant sites in the Himachal Pradesh districts of Chamba, Kullu, and Mandi as a result of research done by Rana and Sharma *et al.* (2020). The objective of this study was to characterize Pseudomonas sp. biochemically and investigate its biocontrol potential against various plant pathogens. They found that these Pseudomonas isolates were highly efficient biocontrol agents and ought to be used in biocontrol applications.

4. Technical program of work:

a. **Survey and assessment of disease samples:** Periodic surveys will be carried out in the pea crop growing areas of mid hill regions of district Mandi of Himachal Pradesh. The survey will be carried out periodically during crop growing seasons. During the course of the survey, diseased pea plants will be collected in labelled paper bags and brought to the laboratory for direct isolation and identification of disease causing pathogens.

b. **Assessment of disease incidence (PDI) and disease severity (PDS):**
For the assessment of disease incidence and disease severity following observation will be recorded. The disease incidence (percentage of plant infected) (PDI) and disease severity (PDS) will be assessed during survey will be recorded periodically in Pea growing areas in different localities. The percent disease incidence and severity will be calculated as per the follows:

$$\text{Plant disease incidence(PDI)} = \frac{\text{Total number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

$$\text{Plant disease severity(PDS)} = \frac{\text{Sum of all diseased ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

2) Field trials:

A field trial will be conducted at research farm of School of Agriculture, Abhilashi University, Mandi (H.P.) to assess the effects of bio-control potential of *Pseudomonas fluorescens* on Pea (*Pisum sativum* L.) in mid hill conditions of Himachal Pradesh. The detail of the treatments will be utilized during present study is as under:

i) Seed Treatments:

Treatment details:

T_1 : Seed treatment by *Pseudomonas fluorescens* + FYM
T_2 : Seed treatment by *Pseudomonas fluorescens* + Vermicompost
T_3 : Seed treatment by *Pseudomonas fluorescens* + Neem cake
T_4 : Seed treatment by *Pseudomonas fluorescens* + Mustard cake
T_5 : Seed treatment by *Pseudomonas fluorescens + Trichoderma viride*
T_6 : Seed treatment by *Pseudomonas fluorescens*
T_7 : Control (no manure or no fertilizer)

ii) Experiment Details:

Design	:	Randomized block design
Variety	:	Golde
Treatments	:	07
Replications	:	03
Experiment year	:	2022-2023
Time of sowing	:	August
Seed rate	:	60 - 80 kg / ha.
Spacing	:	30X10 cm
Total plot	:	21
Net Plot size	:	4 m^2

3. Experimental methods:

a) Biological seed treatment:

Talc base formulation of *Pseudomonas fluorescens* will be used for seed treatment. Seeds treated will be weighed and moistened with tap water. Excess water will be drained. Bio-agents will be added to the seed and mixed gently on seed surface.

b) Sowing of seeds:

The seeds will be sown at about 3 - 4 cm depth by opening furrows with a hand harrow. There will 7 rows in each plot spaced 30 cm apart and plant to plant spacing will be maintained at least 10 cm. Each furrow will be manually dibbled with seed and covered with soil immediately.

c) Weeding:

The crop will be raised mainly as a rainfed crop. Two manual hand weedings will be done for keeping the experimental field free from weeds.

4. Observations to be recorded:

I. Crop studies (Pea) Growth parameters:

a. Rate of germination and survival:

Total number of seeds germinated and survived out of total seed sowed will be calculated and expressed as %age of germination in each treatment and replication.

b. Shoot length:

Height of the plant will be recorded from ground level to the tip of the apical bud. Plant height will be recorded started from germination and thereafter at 30, 60, 90 DAS and so on up to harvesting. For this purpose, ten plants will be selected from each plot and the height of the plant will be measured from ground level to the tip of the topmost fully developed. The average plant height will be expressed in cm.

c. Shoot weight: The shoot weight (fresh and dry weight) will be measured at 30, 60, 90 DAS and so on up to harvesting.

c) Roots length:

Root length will be observed from ground level also and thereafter at 30, 60, 90 days and so on till harvesting after sowing. Ten normal seedlings from every replication will be selected for root measurement. The average of root length will be calculated in cm.

d) Roots weight: The root weight (fresh and dry weight) will be measured at 30, 60, 90 DAS and so on up to harvesting.

II. Yield parameters

a) Number of pods per plant: At the time of maturity, total number of pods per plant will be counted. The average of ten plants will be expressed as the number of pods.

b) Number of seeds per plant: Number of grains will be recorded in each replication and expressed as average number in grains per pod.

c) No. of seeds in 10 g weight: The number of seeds per pod will be weighed from the pods at the time of harvesting.

d) Weight of 100 seeds: The weight 10 seeds will be done after the harvesting of crop. The seed quality will be checked as per the seed obtained at the end.

e) Total yield: The final yield will be calculated on all plants at the time of harvesting of crops. Disease reduction over control will be calculated as follows:

$$\text{Percent yield increase (\%)} = \frac{\text{Yield of treatment} - \text{Yield of control}}{\text{Yield of control}} \times 100$$

III. Crop studies (Pea) disease assessment parameters:

The pea plants get infected with fungal diseases after germination and at different phases of growth will be collected for isolation and identification of the responsible fungal pathogens.

Isolation of soil fungi associated with rhizosphere of pea plants will be carried out at 30, 60, 90 DAS and so on up to harvesting to study the effect of all treatments on soil microflora. For, this serial dilution of the soil will be carried out and appropriate dilution will be inoculated on culture medium. Colony count, identification of fungi to assess their diversity, frequency and abundance will be carried out.

5. Statistical analysis

Data will be recorded on germination percentage, shoot length, root length, number of pods per plant, numbers of grains per pod, disease severity and disease reduction over control. The data will be recorded throughout the cropping period at fixed intervals from the date of appearance of disease till the termination of the experiment. Data will be analyzed by using analysis of variance (ANOVA) in RBD to calculate level of the significance by the magnitude of the F value (P=0.05). (Gomez K A and Gomez A A 1984).

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6. Facilities available

All the necessary facilities required for the conduct of the experiment are available in School of Agriculture, Abhilashi University, Mandi (H.P.).

7. Collaboration with other Departments/Institutes/Universities

Since all the facilities are available within the University, so no collaboration is needed.

CERTIFICATE

Certified that this synopsis of Mrs. Aishwarya, Registration No. AU21AGPhD0076 entitled **"Bio-control potential of *Pseudomonas fluorescens* on Pea (*Pisum sativum* L.) in mid hill conditions of Himachal Pradesh"** has been formulated and finalized by the student him/herself in consultation with the members of the Advisory Committee and is in accordance with the instructions of Dean, Faculty of Agriculture, Abhilashi University, Mandi (H.P.), on the subject.

Signature of the student
(With date)

Signature of the Advisor
(With date)

Advisory Committee

S. No.	Name and Designation		Signature
1.	Dr. Ajay Kumar Gautam Professor (Plant Pathology)	Major Advisor	
2.	Dr. Ritika Singh Assistant Professor (Genetics and Plant Breeding)	Member	
3.	Dr. Ravinder Assistant Professor (Soil Science)	Member	
4.	Mrs. Shivani Kaundal Assistant Professor (Biochemistry and Crop Physiology)	Dean's Nominee	

.....
(PG Coordinator,
School of Agriculture)

APPROVED

.....
(Dean, Faculty of Agriculture)

Dean
Faculty of Agriculture
Abhilashi University, Gohal Chowk
Teh. Chachyot, Distt. Mandi (H.P.) 175028